Sci Amer 1969

Hybrid Somatic Cells

Body cells of different species can now be crossed to form somatic hybrids. This alternative to sexual breeding should facilitate the study of human genetics and provide insights into differentiation

by Boris Ephrussi and Mary C. Weiss

One of the most powerful tools available to the biologist is genetic analysis, through which the structure of the hereditary material and its relation to the functions of cells and organisms are revealed in great detail and with high resolution by the manner in which various characteristics are passed from one generation to the next. At first this analysis was made through sexual breeding, which is easily accomplished in such organisms as the fruit fly *Drosophila* or the mold *Neurospora*. Much of modern genetics, however, rests on information gained by taking advantage of processes that represent alternatives to sexual breeding. These alternatives were first exploited for the genetic analysis of bacteria and viruses. Now such an alternative to sexual breeding has been developed for the genetic analysis of higher animals, including man. The new procedure stems from the discovery that somatic cells (body cells, as opposed to eggs and sperm) can be crossed to form hybrid cells that live and multiply.

The technique was discovered in 1960 by George Barski, Serge Sorieul and Francine Cornefert of the Institut Gustave Roussy in Paris. They had mixed together cultures of two different mouse-cancer cells that could be distinguished by differences in cell morphology and in the shapes of some of their chromosomes, the threadlike structures in which the genes are arranged. After a few months they saw that a few cells of a new type had appeared, containing in a single nucleus the chromosomes of both parents. They were hybrid cells; they had arisen by the fusion of pairs of cells of the two different types. Barski and his colleagues went on to produce pure cultures of these hybrid cells and grow them successfully. Soon other somatic hybrids were produced by crossing various pairs of cell lines.

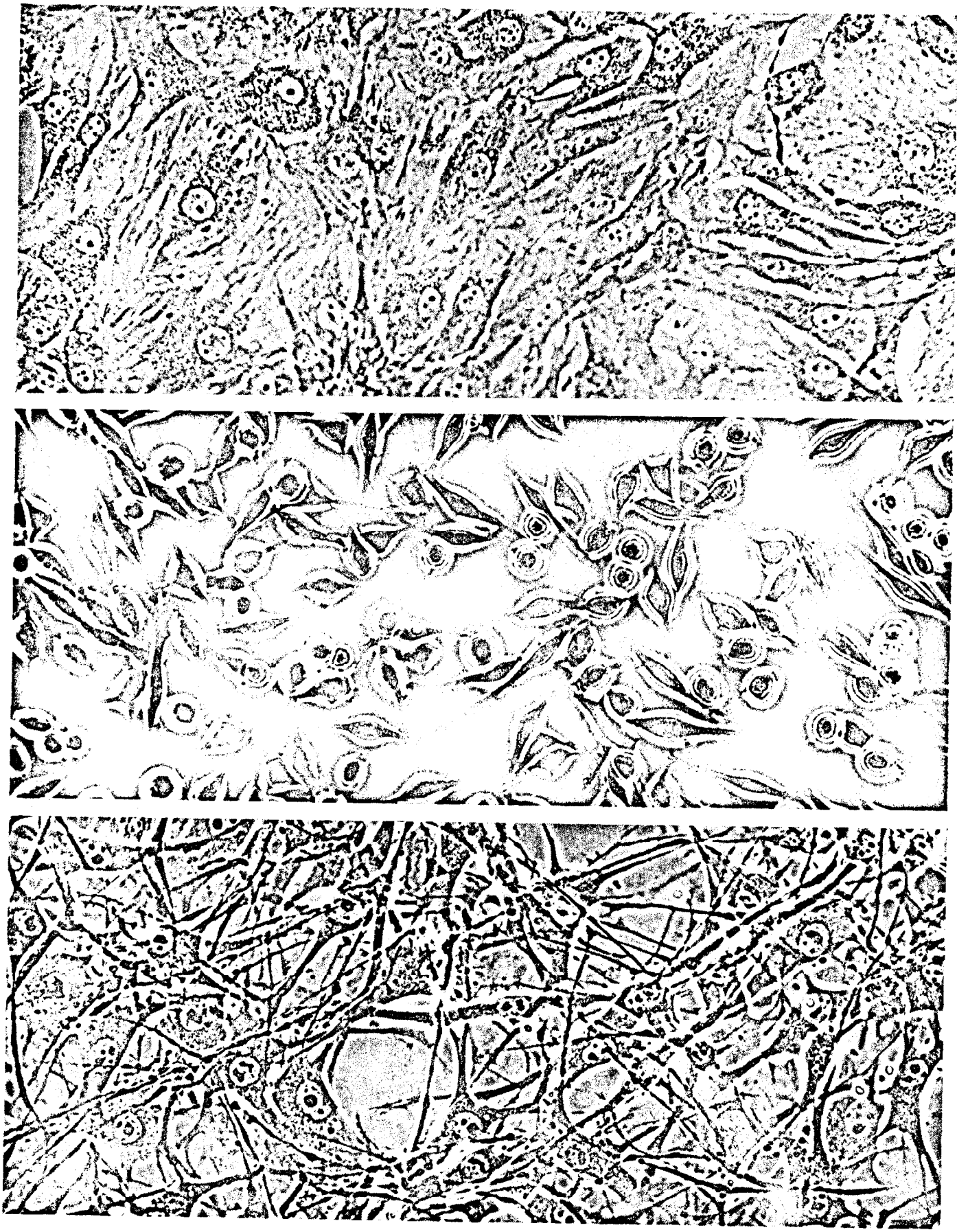
It became clear that the hybrids were not mere curiosities. They have two properties that make them suitable for genetic analysis. First, both sets of chromosomes are functional, and the hybrids therefore exhibit the hereditary characteristics of both parents. Second, as the hybrids multiply they lose some of their chromosomes, and this process produces cells with many different constellations of "parental" genes.

The genetic analysis of hybrid cells has been brought to bear on a number of biological problems. One problem is the formal genetics of higher animals, including man: the location of genes on specific chromosomes. Breeding analysis, which is effective for this purpose in lower animals and plants, is too slow in mammals (even in mice the generation time is about three months), and it is impossible in man. The analysis of somatic cells, on the other hand, proceeds without any breeding of individuals, and the generation time for somatic cells (from one cell division to another) is generally between 12 and 24 hours. Another problem under study is that of cellular differentiation, the process whereby cells that presumably have the same genetic equipment become differently specialized in form and function. To investigate the mechanisms that bring this about one must work with cells that have undergone differentiation; somatic hybridization makes it possible for the first time to apply genetic analysis to differentiated cells. We shall give an account of the first results in both areas of investigation after describing the methods of somatic hybridization and the relevant properties of hybrid cells.

The basic techniques of cell hybridization are those of standard cell culture, in which cells taken from a bit of tissue are allowed to settle out of a suspension and proliferate to form a layer on the bottom of a laboratory dish [*see illustration on page 28*]. To give the cells room to grow the layer is periodically broken up, and a few of the cells are transferred to a new dish; these "serial passages" can be repeated many times, and some cells go on multiplying indefinitely. Such a culture is heterogeneous, having been derived from a fragment containing several cell types. In order to obtain uniform populations of cells one must inoculate a culture dish with a very small number of highly dispersed cells, so that each produces a discrete "clone," or a colony that represents the progeny of a single cell.

Experiments in hybridization begin with a mixed culture of two parent cell lines, each characterized by the presence of marker chromosomes not found in the other. After a few days or weeks of growth one can identify hybrid cells by examining chromosome preparations. The drug colchicine is added to the medium to arrest cell division in metaphase, a stage that is favorable for observation of the chromosomes. The cells are fixed, pipetted onto a slide and stained. Under the microscope such preparations show many normal metaphase chromosome sets of both parental types, each recognizable by the number and shape of the chromosomes. If any hybrid cells have been formed, there will also be hybrid metaphases, recognizable by the large number of chromosomes and by the presence of marker chromosomes from both parents [*see illustration on page 29*].

In Barski's first hybridization experiment, performed in this manner, the hybrids turned out to have a selective advantage over the parental cells and grew

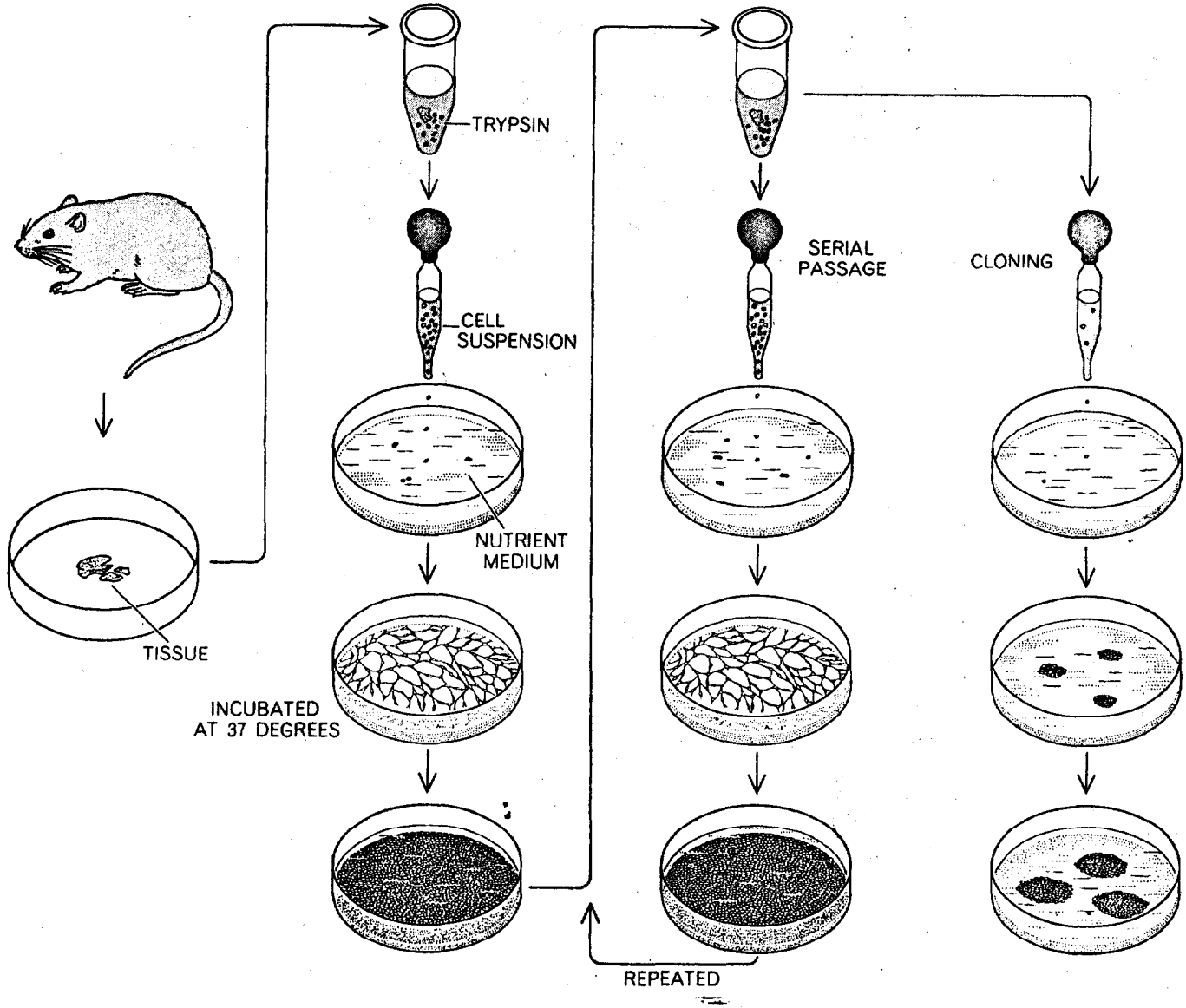


HYBRID CELLS and their parent cells are seen in a set of phase-contrast photomicrographs made by the authors. Rat cells (*top*) and mouse cells (*middle*) are grown together in a cell culture. Some of them fuse and form hybrids, which are isolated and grown in a pure culture (*bottom*). The rat cells tend to flatten out on the surface of the glass culture dish and so they appear thin. The mouse cells attach more loosely to the glass and therefore appear more refractile. The hybrid cells combine the morphological characteristics of both parent lines: they are more refractile than the mouse cells but they attach more completely than the rat cells.

more rapidly, and so they soon constituted a large enough fraction of the total cell population to be isolated by cloning. One of us (Ephrussi), together with co-workers first at the Laboratoire de Génétique Physiologique at Gif in France and then at Western Reserve University, isolated a number of other somatic hybrids. In each case success depended on the hybrid's having a selective advantage in the mixed culture. Since hybrids were rare and it took a long time for enough of them to accumulate so that they could be isolated, and since some crosses were almost surely failing only because the hybrids had no selective advantage, it was clear that a method conferring a decisive selective advantage on hybrid cells would be extremely valuable.

In 1964 John W. Littlefield of the Harvard Medical School devised a system for the selective isolation of hybrid cells [*see illustrations on page 30 and at top of page 31*]. He cultured, in a medium containing the drug aminopterin, two kinds of mutant cells, each of which lacked a different enzyme necessary for growth in the presence of aminopterin. When hybrid cells were formed, they were able to grow in the aminopterin medium by virtue of mutual complementation: each parent supplied the gene for the enzyme the other parent lacked. Littlefield's selective medium therefore kills cells of both parent lines but allows the survival and unhampered growth of hybrid cells. In the course of his experiments Littlefield established that one hybrid cell was produced per 200,000 parental cells (half of each kind).

With Richard L. Davidson, one of us (Ephrussi) then modified Littlefield's method to devise a "half-selective" system that is more generally applicable, in which only one parent cell line lacks one of the enzymes necessary for growth in Littlefield's medium. The other parent can be a normal line, carrying no selective marker, provided that it grows slowly or is inoculated in small numbers. Assume, for example, that a dish is inoculated with a million enzyme-deficient cells and only 100 normal cells. In the selective medium the majority parent degenerates, leaving discrete colonies of the minority parent and of hybrid cells. The hybrids can usually be recognized by their shape, and their hybrid nature



CELL CULTURE begins with minced pieces of tissue from an adult animal or embryo. Incubating the pieces in a digestive enzyme such as trypsin breaks them up into a suspension of single cells. Inoculated into a liquid nutrient medium and incubated, the cells attach to the bottom of the culture dish and divide, producing a continuous sheet. The sheet is again digested with trypsin. If a large fraction of the cells are thereupon inoculated in new medium, the process is repeated. Such serial passages yield heterogeneous cultures because the tissue pieces contained several kinds of cells. If instead a few dispersed cells are inoculated (*right*), they divide to form discrete "clones," or colonies of the progeny of one cell. One clone is then selected with which to start a pure culture.

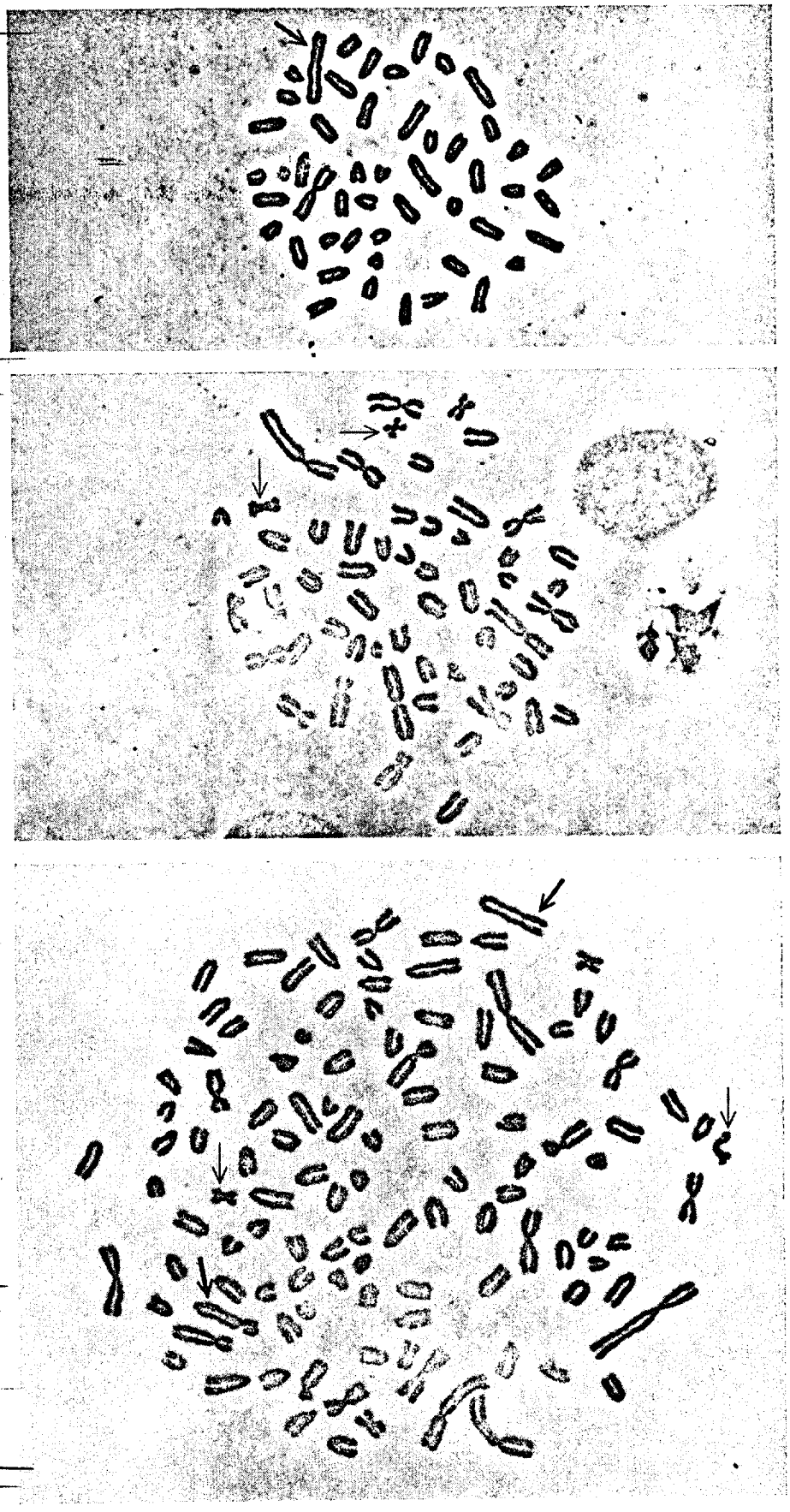
is confirmed by examination of the chromosomes.

These selective systems were first applied to intraspecific crosses (between different cell lines of the same species, usually the mouse). Then we managed to cross cells of different species. The first of these interspecific crosses involved rat and mouse cells; later hamster-mouse hybrids and finally mouse-human hybrids were obtained. Most of the current experimentation is being done with interspecific hybrid cells. The reason is that they fulfill the two requirements for genetic analysis much better than the intraspecific hybrids do.

Since a gene can be recognized only when it mutates to an alternative form that is recognizable in the progeny, the existence of distinguishing genetic markers in the two parents that can be traced in the progeny is a *sine qua non* of genetic analysis. In microbial genetics the most valuable markers have been enzymes that exist in a normal form and also in a form altered by mutation. Such enzyme markers could serve in mammalian-cell studies too, but the trouble is that the necessary mutations are rare and are more difficult to induce in mammalian cultured cells than they are, for example, in bacteria. This paucity of markers led us in 1965 to undertake crosses between different species. We knew that as a consequence of evolution many animal species have come to possess variants of the same enzyme that differ in their structure. These differences affect the physical properties of the enzymes, so that the two variants can be distinguished by such methods as electrophoresis and chromatography. We could expect, therefore, that crossing cells of different species would yield hybrid cells endowed with many built-in enzyme markers.

The only question remaining was whether or not the genes of both parents would in such cases be functional in the hybrids. Fortunately that proved to be the case. For example, in rat-mouse hybrid cells both of the parental forms of the enzyme lactate dehydrogenase (LDH) are synthesized [*see bottom illustration on page 31*]. Malate dehydrogenase and beta-glucuronidase are two other enzymes that have been found in both parental forms in interspecific hybrid cells.

The wealth of genetic markers in interspecific hybrids fulfilled the first prerequisite for genetic analysis. We soon found that the second requirement was fulfilled too: successive generations of



METAPHASE chromosome preparations of the two mouse tumor cells first used in somatic hybridization are shown (*top and middle*) along with a metaphase of the hybrid cell (*bottom*). The chromosomal DNA has been replicated and the two copies of each chromosome are joined to form *V*-shaped or *X*-shaped double chromosomes. The cells of one line (*top*) have two extra-long *V*-shaped chromosomes, one of which is seen in this metaphase (*heavy arrow*). The other line has many *X*-shaped chromosomes (*middle*), two of which are distinctive (*thin arrows*). The hybrid cell (*bottom*) contains the chromosomes of both parent lines.

the interspecific hybrid cells contain decreasing numbers of chromosomes as a result of the loss of chromosomes during cell division. The rate and the extent of the loss vary in different hybrids, but they are generally greater than they are in the intraspecific hybrids.

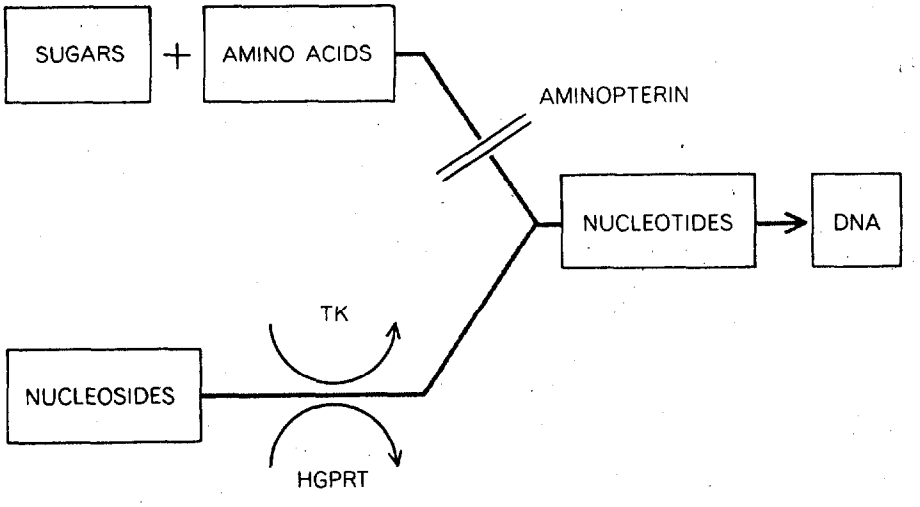
As an example of an intraspecific cross, consider the hybrids derived from two lines of mouse cells. Detailed analysis of the chromosomal changes is not possible because all normal mouse chromosomes are about the same shape and most cultured mouse cells contain only a few marker chromosomes. In general the karyotype, or chromosomal constitution, of hybrid mouse cells is rather stable. There is some loss—as one might expect, since the hybrids contain an excess of most genes and can therefore survive the loss of some chromosomes. The loss usually does not exceed 10 to 20 percent of the number of chromosomes in the original fused nucleus, however, so that even after hundreds of generations the hybrid cells retain most of the chromosomes of both parents.

In contrast to the intraspecific hybrids, those produced by the fusion of cells of different species have a great many marker chromosomes, since there are large differences in the shapes and sizes of the two species' chromosomes [*see illustration on page 32*]. Analysis of the numbers and kinds of chromosomes in successive generations of rat-mouse hybrids showed that although the decrease in the total number of chromosomes is not much greater than it is in mouse-mouse hybrids, the loss is slightly preferential: significantly more of the rat chromosomes than of the mouse chromosomes disappear. In hamster-mouse hybrids there is greater loss of the mouse chromosomes than of the hamster chromosomes.

In 1967 one of us (Weiss) and Howard Green, working at the New York University School of Medicine, succeeded in crossing mouse cells with human cells and found that the hybrids presented an extreme case of preferential chromosome loss. The mouse-human hybrid cells appeared from the beginning to be different from other interspecific hybrids. Instead of having some of the characteristics of each parent, they looked much more like the mouse cells than the human cells [*see illustration on page 34*]. The reason became clear when their karyotype was examined: the cells contained all the expected mouse chromosomes but only from two to 15 of the 46 human chromosomes. Apparently these hybrids had lost most of their human chromosomes soon after being formed. They continued to lose them as they were cultivated. After 100 generations some of the clones had lost all their human chromosomes; the others retained no more than 10 of them. Because it is possible to obtain cells that contain all the mouse chromosomes and either no human ones or very few, the mouse-human hybrids lend themselves, as we shall see, to studies of human genetics.

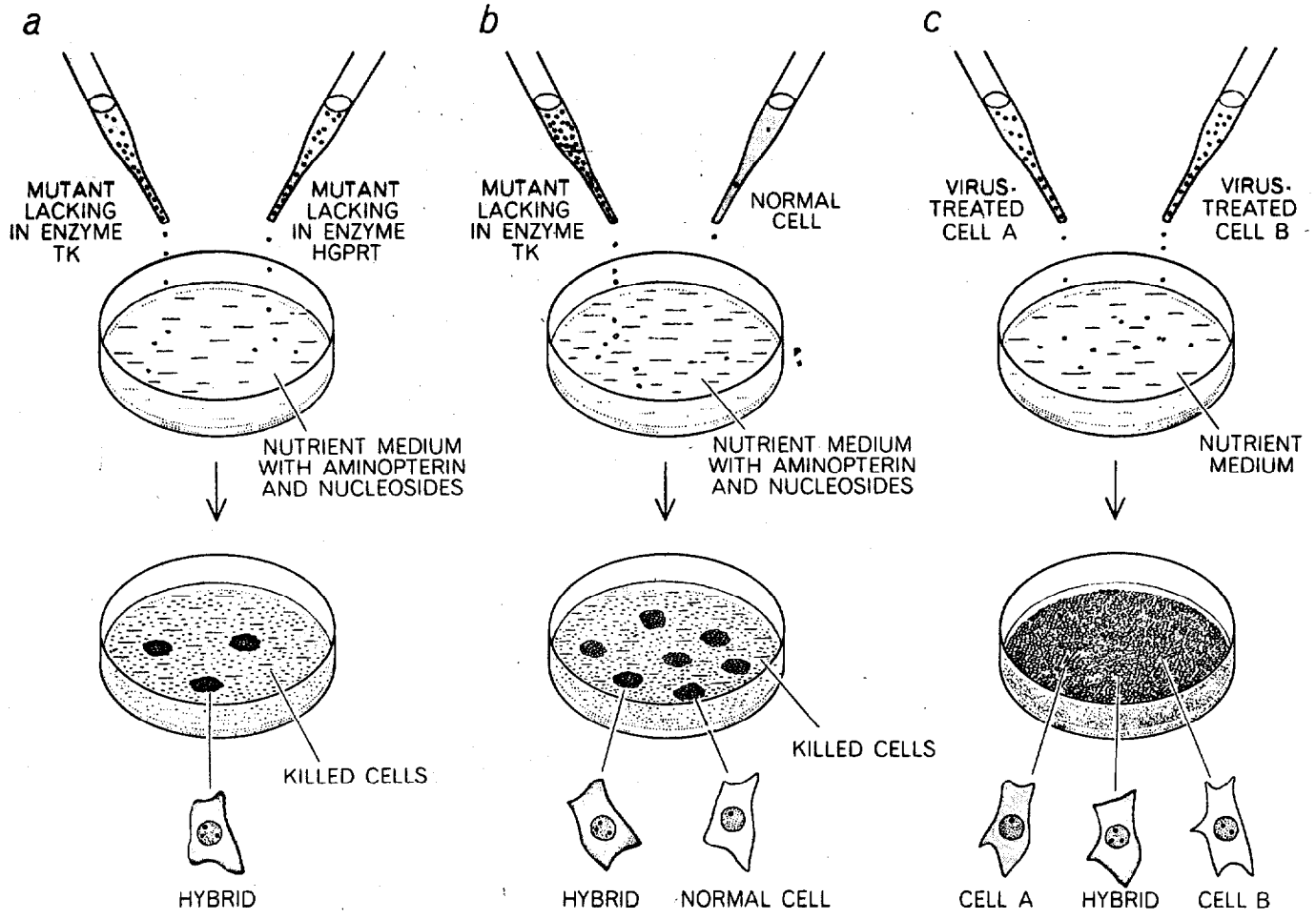
One would like to be able to cross any two cell types at will. Although Littlefield's system and the half-selective system are in principle applicable to a large number of crosses, they depend on the introduction of specific mutations into the cells to be crossed. In mammalian cells this is a difficult and time-consuming process, and for some kinds of cells it may be impossible. During the time it takes to select cells with the mutation resulting in the required enzyme deficiency, for example, other changes may occur that alter other cell properties one would like to retain. There are two ways in which one might bypass this difficulty: either by devising a selective system based on naturally occurring markers or by somehow increasing the frequency of cell fusion to such an extent that the hybrids no longer need to be selected. The second of these approaches has proved to be rewarding.

Some years ago Y. Okada of Osaka University reported that the Sendai strain of parainfluenza virus causes animal cells in suspension to clump together and that many of the clumped cells undergo multiple fusions. With this observation in mind Henry Harris and J. F. Watkins of the University of Oxford were able in 1965 to bring about the fusion of different types of cells with Sendai virus that had been rendered noninfectious by ultraviolet irradiation. The virus treatment induced the formation of giant cells with from two to 10 or more nuclei—in some cases heterokaryons, or cells with nuclei from different parents. Some of these nuclei fused, yielding hybrid karyotypes, but Harris and Watkins saw no hybrids capable of more than a few divisions. Yet the occurrence of nuclear fusion, hybridization and some cell division, and the absence of viral infection (since the virus was inactivated), implied that the method

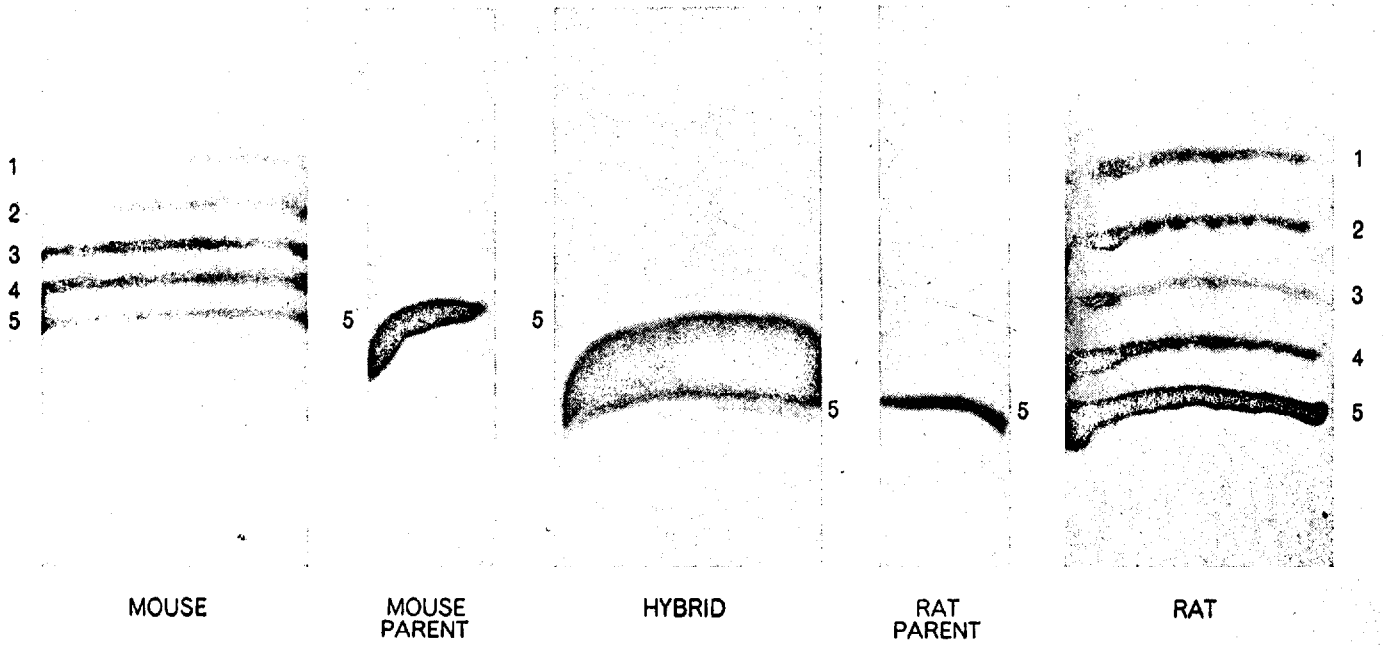


DNA SYNTHESIS from sugars and amino acids is blocked by aminopterin (*top*). An alternative pathway depends on preformed nucleosides (DNA precursors) and the enzymes thymidine kinase (TK) and hypoxanthine guanine phosphoribosyl transferase (HGPRT). Cells with these enzymes can grow in a medium containing aminopterin and nucleosides.

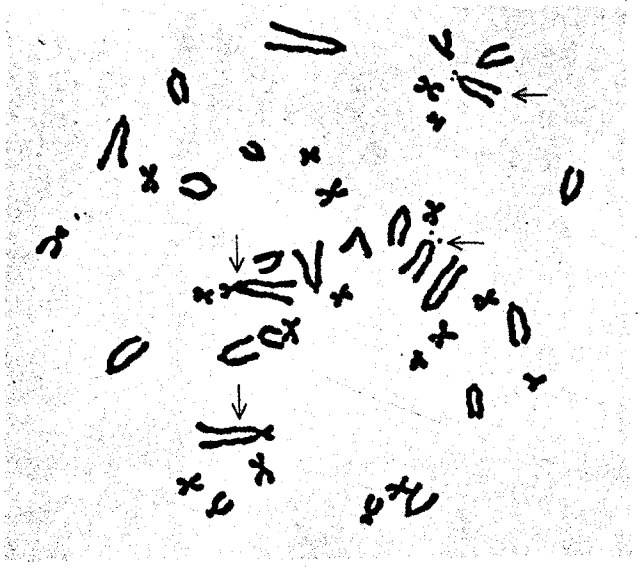
MUTATIONS produce some cells that lack TK but produce HGPRT (*black dots*) and some that lack HGPRT but produce TK (*colored dots*). If such cells are crossed, the hybrid cells contain the genes from both parent lines and therefore produce both parental enzymes.

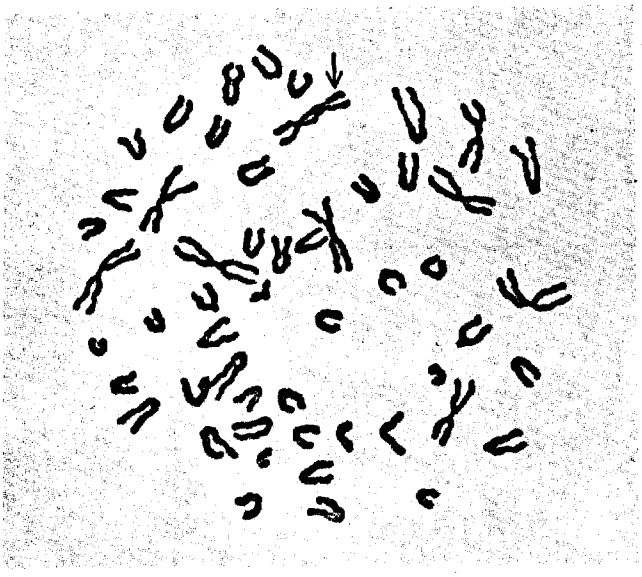


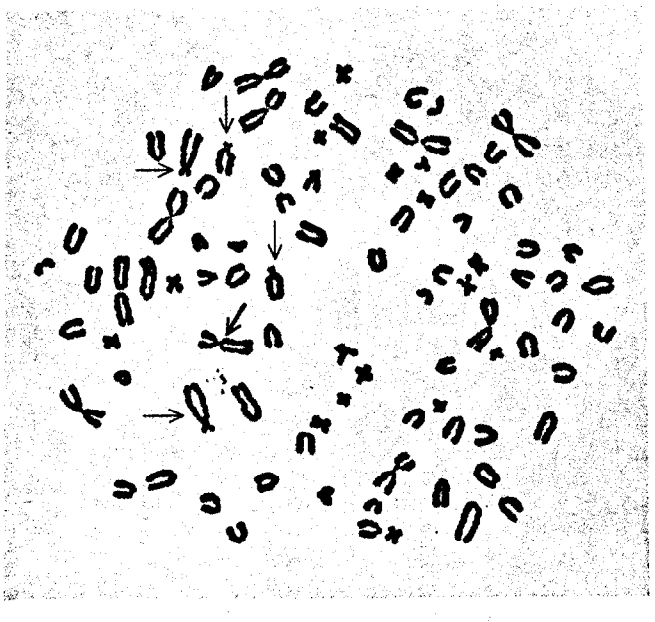
SELECTIVE SYSTEMS are required in order to isolate the rare hybrid cells from the proliferating parent lines. One selective system (*a*) depends on the enzyme activity outlined in the illustration on the opposite page. Cells lacking either enzyme are killed; hybrids, which have both enzymes, live and form colonies (*color*). In the half-selective system (*b*) only one parent cell lacks an enzyme, but since only a few of the other (normal) cells are inoculated, the rare but discrete hybrid colonies can be isolated. In virus-induced hybridization (*c*) any two cells can be crossed. The virus causes them to clump and fuse, promoting hybrid formation.

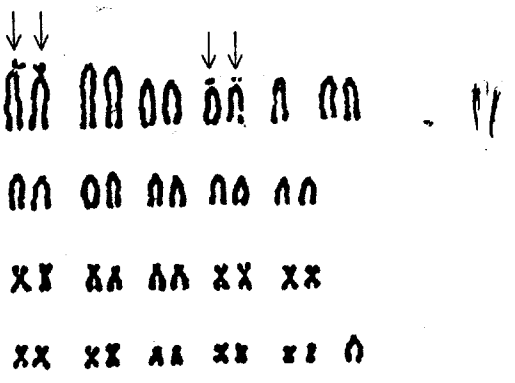


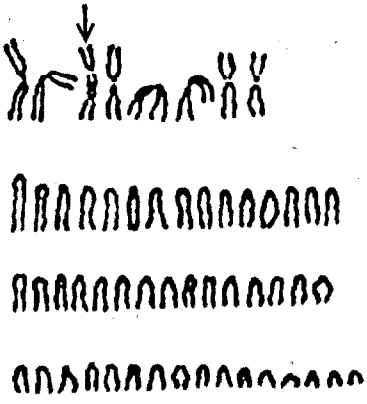
ENZYME MARKERS are available in somatic cells because different species contain slightly different forms of the same enzyme. These forms can be separated on the basis of their mobility in an electric field. Electrophoresis of the LDH found in mouse and rat diaphragm yields five different LDH bands in each (*left and right*). The two No. 5 bands are present in the parental cells used in hybridization. The hybrid has both parental bands and also has three intermediate bands representing hybrid molecules (*center*).

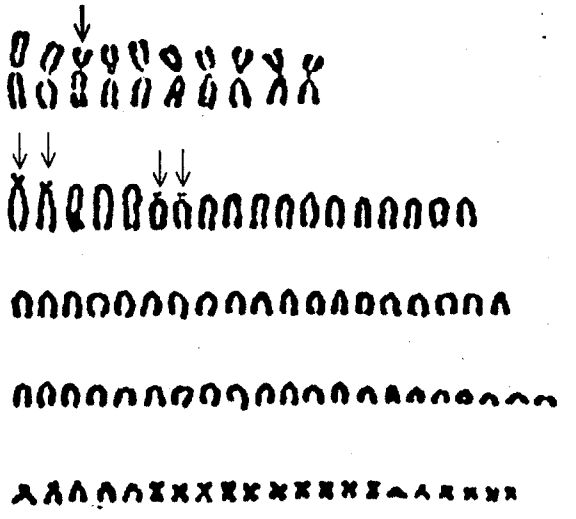












CHROMOSOMES of the rat, mouse and rat-mouse hybrid cells illustrated on page 27 are displayed here in two forms: metaphase figures (*left*) and karyograms (*right*), in which the chromosomes are arranged in groups on the basis of shape and size. The 42 rat chromosomes (*top*) include large ones like unbalanced *X*'s (*thin arrows*) and small *X*-shaped ones. The mouse parental cell (*middle*) has an abnormal number of chromosomes, 54 instead of the usual 40, and a distinctive *X*-shaped one (*heavy arrow*) as well as the usual *V*-shaped ones. The hybrid from which this preparation was made (*bottom*) has 89 chromosomes, of both parental types.

could be developed to provide large numbers of hybrids capable of prolonged multiplication.

This was achieved by George Yerganian and M. B. Nell of the Children's Cancer Research Foundation in Boston and then by Hayden Coon and one of us (Weiss) at the Department of Embryology of the Carnegie Institution of Washington. In the latter case the cell lines involved were such that the frequency of virus-induced hybrids could be compared with that of spontaneous hybrids isolated by the selective techniques, and so it was possible to determine the effectiveness of the virus in promoting hybridization. Viable hybrids appeared from 100 to 1,000 times more frequently in cultures treated with inactivated virus than they did in cultures left to spontaneous hybridization. The virus-induced hybrids proved to have the same properties as the spontaneous ones and can therefore be used for the same kinds of experiments. It is now possible to make crosses between cells to which no selective system is applicable, and this should mean that almost any two cells can be crossed and the resulting hybrid can be isolated.

One of the most interesting findings of somatic-hybridization studies is the very fact that somatic cells of different origins are compatible. The incompatibility between the sperm of one species and the egg of another is well established; in extreme cases an egg fertilized by a sperm of another species immediately expels the nucleus of the sperm. It is therefore surprising to see that the nuclei of cells of two different species fuse and in most cases at once begin to function harmoniously. This implies that the intracellular signals that dictate the sequence of biochemical events in one parent's division cycle must be "understood" by the components of the other cell—in spite of the millions of years during which mammalian species have diverged from their common ancestors by accumulating gene mutations.

A related finding is that the hybrid cells synthesize hybrid enzymes that function satisfactorily. As we mentioned before, rat-mouse hybrids synthesize both parental forms of the enzyme LDH. We have found, moreover, that in the hybrids some of the active enzyme molecules, which are composed of four subunits, are themselves hybrid in nature, formed by the random association of rat subunits and mouse subunits. Several other examples of hybrid enzyme molecules have been reported in interspecific hybrids; since many enzymes are composed of subunits, molecules of this kind are probably common in hybrid cells. The existence of functional hybrid enzymes was not in itself a surprise. Clement L. Markert of Johns Hopkins University had earlier produced such LDH molecules by chemical methods. What is surprising is to find that homologous genes that have diverged as widely as is suggested by the different structures of the enzymes they specify can still produce proteins similar enough to associate into molecules whose enzyme activity can fully satisfy the requirements of a living cell.

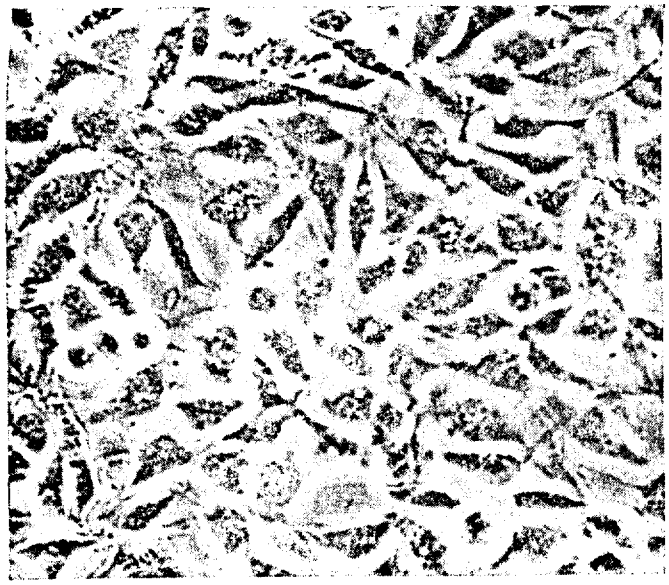
The two examples we shall give of current experiments utilizing somatic hybridization have to do with the formal genetics of man and with the study of gene expression and its control in cellular differentiation.

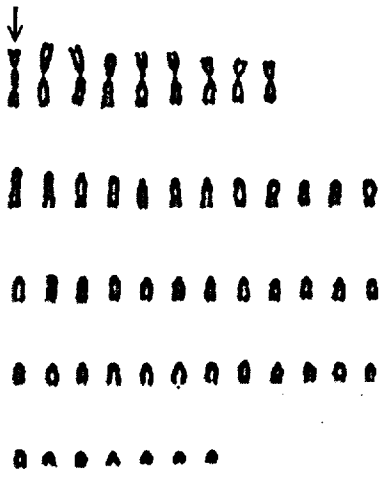
Study of the formal genetics of any organism begins with the determination of linkage groups and the assignment of genes to specific chromosomes. Then genes are localized in specific segments of the chromosomes, establishing genetic maps for the species. The required data are ordinarily obtained by sexual breeding over many generations, each involving segregation and recombination: the processes, which occur during formation of the germ cells, by which the various parental genes are distributed in different assortments to different daughter cells, resulting in progeny with a range of different characteristics. In effect, the loss of chromosomes by successive generations of somatic hybrid cells takes the place of the segregation and recombination that occur in germ cells, making it possible to begin to determine human linkage groups.

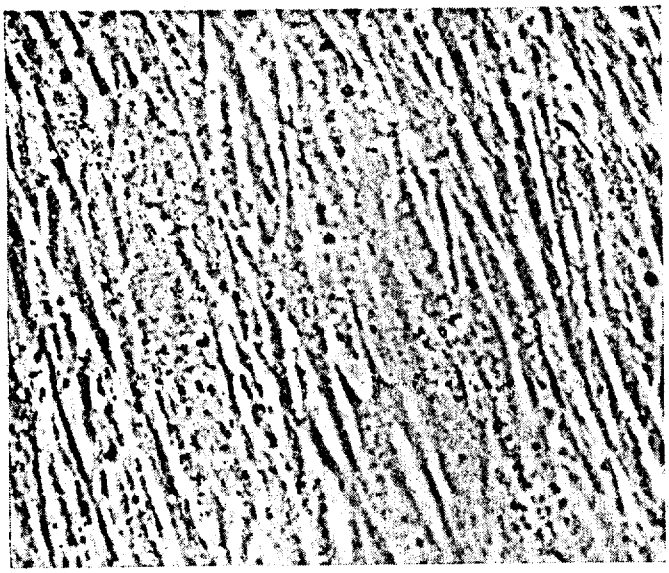
Mouse-human hybrids eventually lose all their human chromosomes. By studying them at a stage when they contain only a few, one can correlate the loss of a specific human gene product with the loss of a specific chromosome. So far this has been done for one enzyme. The mouse cells Weiss and Green used were deficient in thymidine kinase, one of the enzymes required for growth in Littlefield's medium. The survival and growth of hybrid cells maintained in the selective medium therefore depend on the presence of the human gene for thymidine kinase. In several clones of hybrid cells only from one to three human chromosomes remained after 100 to 150 generations [*see illustration on page* 35]. In each of them one specific human chromosome was still present: a small one of the group designated *E*. Presumably this chromosome carries the thymidine kinase gene. This was confirmed when the clones were removed from the selective medium and exposed to bromodeoxyuridine, which kills cells containing thymidine kinase. None of the cells that survived this treatment contained the chromosome in question.

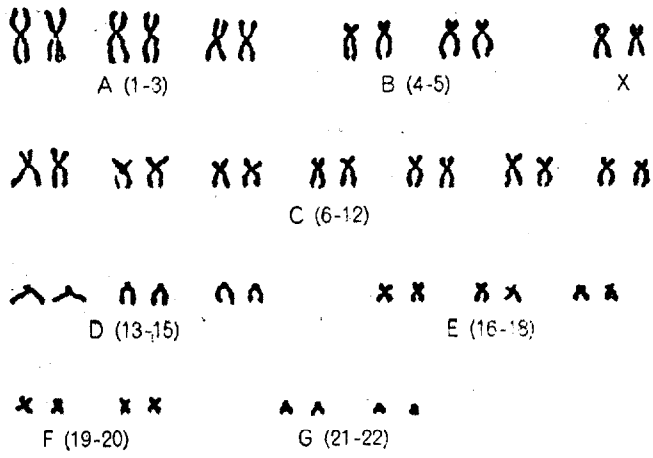
The mouse-human hybrids can surely be used to locate other genes in specific chromosomes. The gene need not be for an enzyme that is missing from the mouse parent as in the case of thymidine kinase. Because of the physical differences between mouse and human forms of many enzymes, one can find out which human enzymes are retained in various hybrid populations and so correlate the presence of the enzyme with that of a specific chromosome. Similar experiments should reveal the location in human chromosomes of genes that determine the presence of antigens. Somatic hybridization has already shown that genes for human antigens must be widely distributed among the human chromosomes since the human antigenic activity of the hybrid cells is proportional to the number of human chromosomes they contain. Adding a purified antiserum that acts against a specific antigen should make it possible to trace that antigen's gene to a single chromosome. In short, it appears likely that within a few years a number of human genes will have been assigned to specific chromosomes. Whether more refined genetic analysis and mapping will be possible with somatic hybrids depends on whether genetic events comparable to those that produce recombination within the chromosomes of germ cells occur also in mammalian somatic cells.

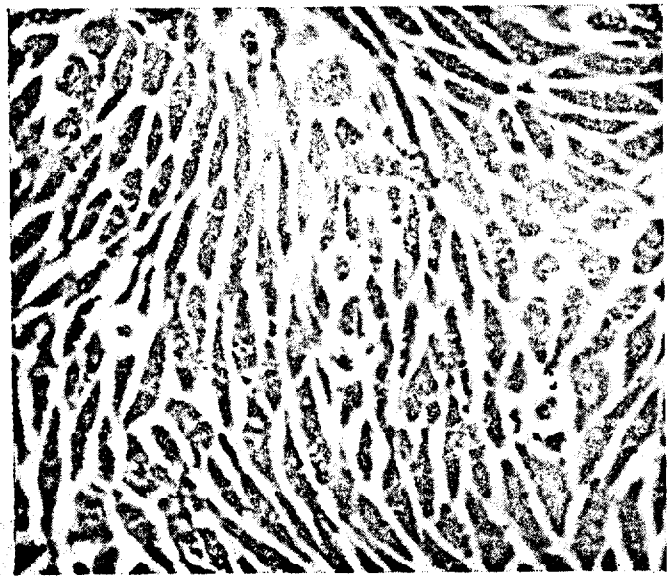
Crosses between differentiated and undifferentiated cells, or between differently differentiated cells, can provide information on the nature of the regulatory processes involved in cell specialization. The activities of somatic cells can be divided into two general categories. There are "essential" functions that are indispensable for the cells' own maintenance and growth and there are specialized "luxury" functions, such as the formation of muscle fiber, the secretion of hormones and the production of pigment, that are necessary for the survival of the organism but not for the survival of isolated cells. The essential functions are expressed in all growing somatic cells. Each luxury function, on the other hand, is expressed in a different line of specialized cells. In 1961

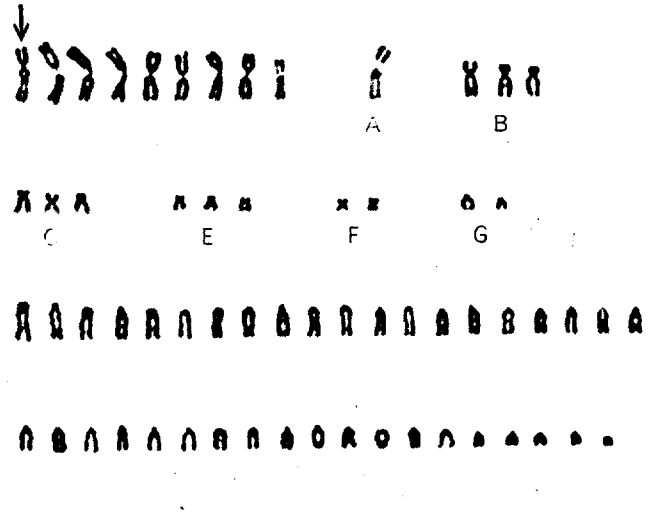












MOUSE-HUMAN hybrids are illustrated by the cell cultures (*left*) and the karyograms (*right*) of the mouse parent line (*top*), the human parent (*middle*) and the hybrid (*bottom*). The human cells, derived from embryonic lung tissue, contain the normal number of chromosomes (46, or 23 pairs), arranged here in the usual seven groups (plus the two female sex chromosomes). Except for a tendency to align in parallel, the hybrid cells look more like the mouse cells than the human ones. This is in keeping with the fact that the hybrid karyogram contains only 14 of the 46 human chromosomes, which are readily distinguished from mouse chromosomes.

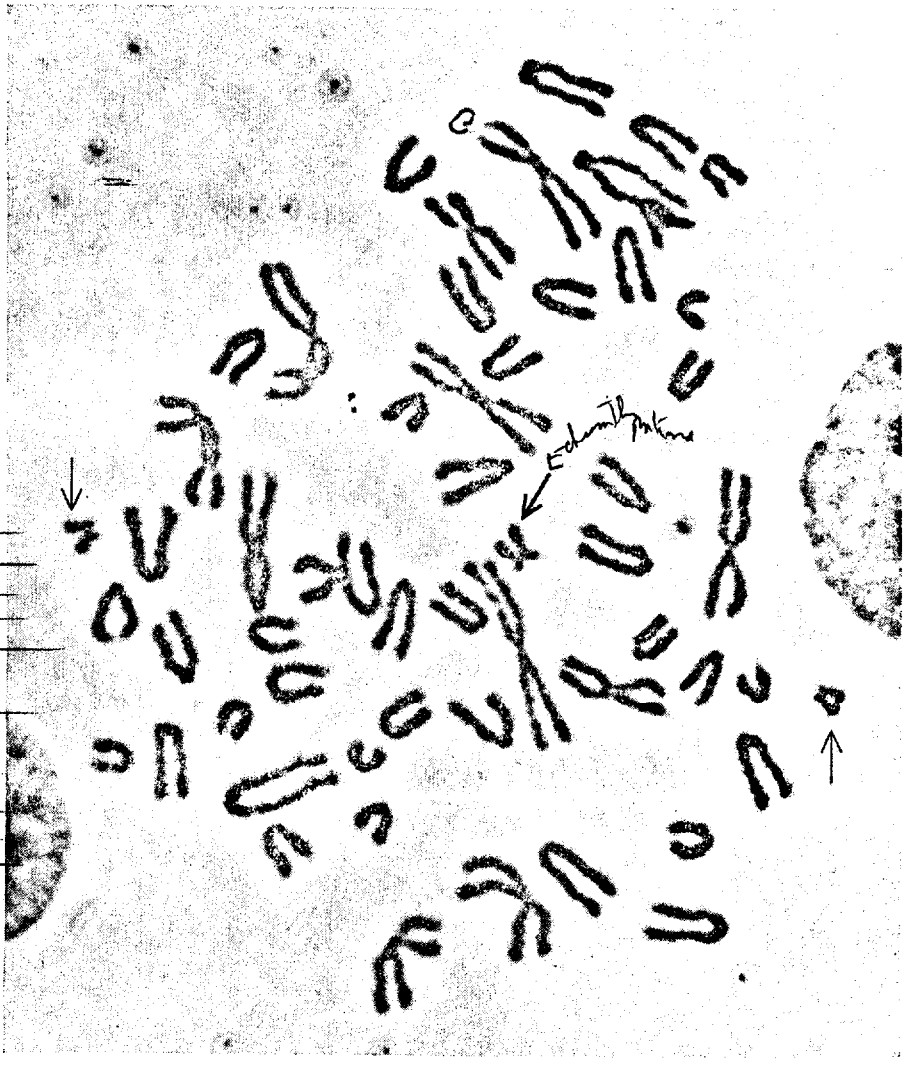
François Jacob and Jacques Monod of the Pasteur Institute in Paris discovered mechanisms that regulate the activity of genes in bacteria. It is generally believed that similar mechanisms are responsible for specialization of somatic cells—that sets of genes governing the various luxury functions are activated or repressed as required in the cells of the different tissues. Since bacterial and mammalian cells have very different properties and requirements, however, it would not be surprising if somatic cells have evolved some unique mechanisms for the regulation of gene activity.

Working with Davidson and Kohtaro Yamamoto at Western Reserve, one of us (Ephrussi) crossed two cells, one of which synthesizes a certain luxury product. This differentiated parent is a line of hamster tumor cells that produce melanin, a dark pigment. When cells of this melanoma line are crossed with cells of a number of different mouse lines that have never produced melanin, the hybrids have a rather stable karyotype and contain most of the chromosomes of both parents. Yet among the many hybrid colonies obtained, none synthesizes melanin or contains the enzyme dopa oxidase, which is required for the synthesis of the pigment and which is present in large quantities in the melanoma cells. Obviously, when the melanoma cells fuse with the unpigmented ones, the synthesis of the enzyme is halted by some regulatory substance produced by the normal cells. Whether this block occurs at the level of the gene specifying dopa oxidase (which was active in the melanoma cell) or at some later step in the process leading to dopa oxidase production is something that remains to be determined.

Some light may be shed on the mechanism of regulation of gene activity in mammalian cells by examination of highly segregated hybrids between melanoma cells and cells that do not produce the pigment. If the genes responsible for producing melanin are active in the melanoma parent because of some stable change at the chromosome level, and if they are only temporarily repressed in the hybrid cells, then the loss of the repressing chromosomal material should bring a resumption of melanin synthesis. If synthesis does not resume, one will have to conclude that the continuous production of melanin in the melanoma parent was not due to a stable cellular change in the first place.

The melanoma cell is a differentiated cell that synthesizes both a specialized product and all the enzymes necessary for continuous and rapid growth. Some other highly differentiated cells no longer grow at all; the essential syntheses that occur in growing cells are arrested. Does this reflect a total and definitive inactivation of the nucleus associated with extreme differentiation? Harris and his co-workers have shown that it does not. They produced heterokaryons between nucleated red blood cells taken directly from the blood of a hen and actively growing, undifferentiated human cells. The human cells are characterized by rapid synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA); the hen blood cells synthesize neither and they have small, highly condensed nuclei characteristic of quiescent cells. In the heterokaryons the red-cell nuclei undergo dramatic changes: they swell up, their chromosomal material becomes less condensed and they resume the synthesis of both DNA and RNA. Clearly the red-cell nucleus is reactivated; it must not have been irreversibly inactivated to begin with.

It will be most interesting to determine precisely which of the numerous possible functions are resumed in these reactivated nuclei. Can the nuclei of highly differentiated cells such as the red blood cells be induced to perform not only some essential functions but also specialized functions characteristic of other differentiated cell types? It should be possible to answer this question by determining the nature of the products synthesized by red-cell nuclei that are reactivated by fusion with other differentiated cells. A positive answer would open a new line of biochemical investigation of the factors that control cellular differentiation in embryonic development.



ENZYME AND CHROMOSOME can be correlated in the clone from which this metaphase was taken. All but three human chromosomes have been lost: two of Group *G* (*thin arrows*) and one of Group *E* (*heavy arrow*) remain. The Group *E* chromosome is always retained in clones grown, as this one was, in a medium in which cells survive only if they produce the enzyme TK. Therefore that chromosome must carry the gene for TK.

BIBLIOGRAPHY

Readers interested in further reading on the subjects covered by articles in this issue may find the lists below helpful.

THE DYNAMICS OF THE ARMS RACE

THE EFFECTS OF NUCLEAR WEAPONS. Edited by Samuel Glasstone. United States Department of Defense and the United States Atomic Energy Commission, April, 1962.

DEBATE: THE ANTIBALLISTIC MISSILE. Edited by Eugene Rabinowitch and Ruth Adams. Bulletin of the Atomic Scientists, 1968.

THE ESSENCE OF SECURITY. Robert S. McNamara. Harper & Row, Publishers, 1968.

THE FUTURE OF THE STRATEGIC ARMS RACE: OPTIONS FOR THE 1970's. George W. Rathjens. Carnegie Endowment for International Peace, 1969.

HYBRID SOMATIC CELLS

HYBRIDIZATION OF SOMATIC CELLS AND PHENOTYPIC EXPRESSION. Boris Ephrussi in *Developmental and Metabolic Control Mechanisms and Neoplasia: A Collection of Papers Presented at the Nineteenth Annual Symposium on Fundamental Cancer Research, 1965*. The Williams and Wilkins Company, 1965.

ARTIFICIAL HETEROKARYONS OF ANIMAL CELLS FROM DIFFERENT SPECIES. H. Harris, J. F. Watkins, C. E. Ford and C. I. Schoefl in *Journal of Cell Science*, Vol. 1, No. 1, pages 1–31; March, 1966.

STUDIES OF INTERSPECIFIC (RAT × MOUSE) SOMATIC HYBRIDS. I: ISOLATION, GROWTH AND EVOLUTION OF THE KARYOTYPE. II: LACTATE DEHYDROGENASE AND β-GLUCURONIDASE. Mary C. Weiss and Boris Ephrussi in *Genetics*, Vol. 54, No. 5, pages 1095–1109, 1111–1122; November, 1966.

HUMAN-MOUSE HYBRID CELL LINES CONTAINING PARTIAL COMPLEMENTS OF HUMAN CHROMOSOMES AND FUNCTIONING HUMAN GENES. Mary C. Weiss and Howard Green in *Proceedings of the National Academy of Sciences*, Vol. 58, No. 3, pages 1104–1111; September 15, 1967.

EIDETIC IMAGES

THE EIDETIC CHILD. Heinrich Klüver in *A Handbook of Child Psychology*, edited by Carl Murchison. Clark University Press, 1931.

EIDETIC IMAGERY, I: FREQUENCY. Ralph Norman Haber and Ruth B. Haber in *Perceptual and Motor Skills*, Vol. 19, pages 131–138; 1964.

EIDETIC IMAGERY: A CROSS-CULTURAL WILL-O'-THE-WISP? Leonard W. Doob in *The Journal of Psychology*, Vol. 63, pages 13–34; 1966.

THE SYNTHETIC ELEMENTS: IV

MAN-MADE TRANSURANIUM ELEMENTS. Glenn T. Seaborg. Prentice-Hall, Inc., 1963.

SYNTHETIC TRANSURANIUM ELEMENTS. Earl K. Hyde. U.S. Atomic Energy Commission, Division of Technical Information, December, 1964.

ELEMENTS BEYOND 100, PRESENT STATUS AND FUTURE PROSPECTS. Glenn T. Seaborg in *Annual Review of Nuclear Science*, Vol. 18, pages 53–152; 1968.

THE ENCYCLOPEDIA OF THE CHEMICAL ELEMENTS. Edited by Clifford A. Hampel. Reinhold Book Corporation, 1968.

MASS PRODUCTION AND PRACTICAL APPLICATIONS OF ACTINIDE ELEMENTS. Glenn T. Seaborg in *Isotopes and Radiation Technology*, Vol. 6, No. 1, pages 1–18; Fall, 1968.

STONE TOOLS AND HUMAN BEHAVIOR

LE PALÉOLITHIQUE INFÉRIEUR ET MOYEN DE JABRUD (SYRIE) ET LA QUESTION DU PRÉ-AURIGNACIEN. F. Bordes in *L'Anthropologie*, Vol. 59, Nos. 5–6, pages 486–507; 1955.

THE HUMAN USE OF THE EARTH. Philip L. Wagner. The Free Press of Glencoe, Illinois, 1960.

ME'ARAT SHOVAKH (MUGHARET ES-SHUBBABIQ). Sally R. Binford in *Israel Exploration Journal*, Vol. 16, No. 1, pages 18–32, No. 2, pages 96–103; 1966.

A PRELIMINARY ANALYSIS OF FUNCTIONAL VARIABILITY IN THE MOUSTERIAN OF LEVALLOIS FACIES. Lewis R. Binford and Sally R. Binford in *American Anthropologist*, Vol. 68, No. 2, Part 2, pages 238–295; April, 1966.

MODERN FACTOR ANALYSIS. Harry H. Harman. The University of Chicago Press, 1967.

SOIL POLLUTANTS AND SOIL ANIMALS

EFFECTS OF PESTICIDE RESIDUES ON SOIL INVERTEBRATES AND PLANTS. C. A. Edwards in *Ecology and the Industrial Society: A Symposium of the British Ecological Society Swansea 13–16 April 1964*, edited by Gordon T. Goodman, R. W. Edwards and J. M. Lambert. Blackwell Scientific Publications, 1965.

INSECTICIDE RESIDUES IN SOILS. C. A. Edwards in *Residue Reviews: Vol. XIII*, edited by Francis A. Gunther. Springer-Verlag, 1966.

SOME EFFECTS OF CHLORFENVINPHOS, AN ORGANOPHOSPHORUS INSECTICIDE, ON POPULATIONS OF SOIL ANIMALS. C. A. Edwards, A. R. Thompson and K. I. Beynon in *Revue d'Ecologie et de Biologie du Sol*, Vol. 5, No. 2, pages 199–224; April, 1968.

STEAM TURBINES

STEAM TURBINES. Edwin F. Church, Jr. McGraw-Hill Book Company, Inc., 1950.

STEAM TURBINES AND THEIR CYCLES. J. Kenneth Salisbury. John Wiley & Sons, Incorporated, 1950.

STEAM TURBINE PERFORMANCE AND ECONOMICS. Robert L. Bartlett. McGraw-Hill Book Company, Inc., 1958.

HORNS AND ANTLERS

HISTOGENESIS OF BONE IN THE GROWING ANTLER OF THE CERVIDAE. Walter Modell and Charles V. Noback in *The American Journal of Anatomy*, Vol. 49, No. 1, pages 65–95; September 15, 1931.

THE POST-NATAL DEVELOPMENT OF THE HORN TUBULES AND FIBRES (INTERTUBULAR HORN) IN THE HORNS OF SHEEP. A. N. George in *The British Veterinary Journal*, Vol. 112, No. 1, pages 30–34; January, 1956.

STRUCTURE OF RHINOCEROS HORN. M. L. Ryder in *Nature*, Vol. 193, No. 4821, pages 1199–1201; March 24, 1962.

TROPHIC RESPONSES TO TRAUMA IN GROWING ANTLERS. Anthony B. Bubenik and R. Pavlansky in *The Journal of Experimental Zoology*, Vol. 159, No. 3, pages 289–302; August, 1965.

THERMOREGULATORY FUNCTION OF GROWING ANTLERS. Bernard Stonehouse in *Nature*, Vol. 218, No. 5144, pages 870–872; June 1, 1968.

THE AMATEUR SCIENTIST

AERODYNAMICS OF SAILS AND RACING TACTICS. Manfred Curry. Charles Scribners Sons, 1924.

YOUR BOOK OF KITES. Clive Hart. Faber and Faber, 1964.

Established 1845 **SCIENTIFIC AMERICAN** April 1969 Volume 220 Number 4

ARTICLES

DEPARTMENTS

PUBLISHED MONTHLY BY SCIENTIFIC AMERICAN, INC., 415 MADISON AVENUE, NEW YORK, N.Y. 10017. SECOND-CLASS POSTAGE PAID AT NEW YORK, N.Y., AND AT ADDITIONAL MAILING OFFICES. AUTHORIZED AS SECOND-CLASS MAIL BY THE POST OFFICE DEPARTMENT, OTTAWA, CANADA, AND FOR PAYMENT OF POSTAGE IN CASH. SUBSCRIPTION RATE: $6 PER YEAR.